

HYBRIDIZATION OF *TAMARIX RAMOSISSIMA* AND *T. CHINENSIS*
(SALT CEDARS) WITH *T. APHYLLA* (ATHEL) (TAMARISK) IN THE
SOUTHWESTERN USA DETERMINED FROM DNA
SEQUENCE DATA

JOHN F. GASKIN

U.S. Department of Agriculture, Agricultural Research Service, Northern Plains
Agricultural Research Lab, P.O. Box 463, Sidney, MT 59270
jgaskin@sidney.ars.usda.gov

PATRICK B. SHAFROTH

U.S. Geological Survey, Fort Collins Science Center, 2150 Centre Ave.,
Building C, Ft. Collins, CO 80526

ABSTRACT

Morphological intermediates between *Tamarix ramosissima* or *T. chinensis* (saltcedars) and *T. aphylla* (athel) were found recently in three locations in the southwestern USA, and were assumed to be hybrids or a previously unreported species. We sequenced chloroplast and nuclear DNA from putative parental and hybrid morphotypes and hybrid status of morphological intermediates was supported. Chloroplast data suggest that the seed source for these hybrids is *T. aphylla*. Invasive *T. aphylla* genotypes found in Australia match those found in the USA. Seed was collected from one of the hybrids, and a low percentage of it was viable. This hybrid combination has not been previously reported in the USA or the native ranges of the species. Although populations of this novel *Tamarix* hybrid appear to be uncommon at present, both parental species are considered invasive (saltcedars in North America; athel in Australia), and it is possible that more aggressive hybrid genotypes could be produced. Therefore, natural resource managers concerned with the potential spread of non-native species should be aware of the existence of these plants and monitor their future spread.

Key Words: hybridization, *Tamarix*, tamarisk, saltcedar, athel, invasive.

Accidental and deliberate importations of plants can place historically allopatric species in close proximity (U.S. Congress OTA 1993), which can lead to novel hybridization. These hybrid events may stimulate the evolution of invasiveness in plants (Ellstrand and Schierenbeck 2000) by providing new opportunities for genetic variation and evolutionary novelty (Stebbins 1969) including hybrid traits that exceed those found in the parental types (e.g., Rieseberg et al. 1999). The considerable lag time that can exist between establishment of a naturalized population and its subsequent spread into new areas (often decades or more; Kowarik 1995; Louda et al. 1997), combined with potentially deleterious ecological effects (Vila et al. 2000), emphasizes the need for vigilance over novel hybrids involving naturalized species.

The Old World plant genus *Tamarix* L. (Tamaricaceae) contains about 54 species of shrubs and trees (Baum 1978). Multiple *Tamarix* species were brought to the USA from southern Europe and Asia during the 1800's to be used for shade and erosion control (Baum 1967). The western USA now contains large-scale invasions totaling 470,000–650,000 hectares (Zavaleta 2000) of *T. ramosissima* Ledeb. and *T. chinensis* Lour. (common names “saltcedar” or “tamarisk”). Hybrids of these two species are the most common genotype

in the invasion (Gaskin and Schaal 2002). The two species are morphologically very similar, and hybridization has probably added to their taxonomic confusion.

Another species, *T. aphylla* (L.) H. Karst. (common name “athel”), is native to extreme northern Africa and southwestern Asia (Baum 1978) and has been planted in the USA as a shade and wind break tree but has not spread extensively (Meyers-Rice 1997). In Australia, athel was also imported as a shade tree (perhaps from California in the 1930's (Fuller 1998)), and did not spread for decades until the mid-1970's when large scale invasions occurred, most notably on 400 km of the Finke River in central Australia (Griffin et al. 1989). Oddly, *T. ramosissima* is also naturalized in Australia (Thorpe and Lynch 2000), but unlike *T. aphylla*, has not spread extensively and is not considered a Weed of National Significance. The reason for the historically different behavior of *T. ramosissima* and *T. aphylla* in Australia compared to the USA is unknown, but the recent discovery of thousands of naturalized athels propagated from seed along the shore of Lake Mead, NV (Barnes 2003) suggests that athel has some potential to become invasive in the USA.

Many *Tamarix* species are difficult to distinguish using morphology, especially the saltcedars *T. ra-*



4–15 stamens, but *T. aphylla*, *T. ramosissima* and *T. chinensis* are extremely similar, each with a pentamerous calyx, corolla, and androecium (Baum 1978).

Within the last few years three populations have been found in the southwestern USA containing leaf morphologies that are intermediate to *T. ramosissima* or *T. chinensis* (hereafter referred to as saltcedars) and *T. aphylla* (hereafter referred to as athel), suggesting that they may be a novel hybrid, or a species of *Tamarix* previously unreported in the USA. Here, we genetically characterize the morphologically intermediate plants from the three populations using chloroplast and nuclear DNA sequence markers to determine their identity. We also genetically compare invasive specimens of athel from Australia with newly invasive athel specimens from the USA.

METHODS

Study Areas

FIG. 1. Leaf morphology of saltcedars (*Tamarix ramosissima* and *T. chinensis*; sessile leaves), athel-saltcedar hybrid (strongly clasping leaves), and athel (*T. aphylla*; vaginate leaves).

mosissima and *T. chinensis* (Crins 1989; Gaskin and Schaal 2003). However, *T. aphylla* is very distinct from *T. ramosissima* and *T. chinensis* due to its vaginate (completely sheathing the stem) instead of sessile leaves (Fig. 1) and its tree habit under favorable conditions, compared to a large shrub habit. The floral morphology within the genus *Tamarix* can vary from 4–5 sepals, 4–5 petals, and

The populations that are morphologically intermediate to athel and saltcedar are known from only three locations (Fig. 2). One is within a stand dominated by athel on Boulder Beach, Lake Mead, NV (ca. 36°2'46"N lat., 114°48'24"W long., elev. 370 m). The area also contains numerous saltcedars closer to the lake edge. The number of intermediates present at Boulder Beach is unknown, but we found five in an informal survey of a small portion (ca. 1.0 ha) of the athel stand.

The second area containing morphologically intermediate plants is near Walter's Camp along the Colorado River, ca. 40 km south of Blythe, CA (ca. 33°15'9"N lat., 114°41'48"W long., elev. 63 m).

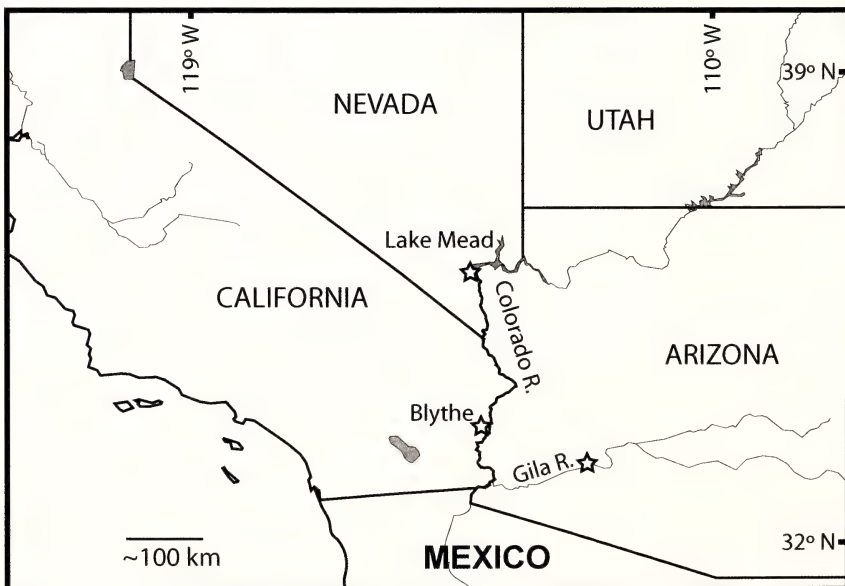


FIG. 2. Locations of *Tamarix* populations containing hybrids between athel and saltcedar (indicated by stars).

TABLE 1. MORPHOLOGICAL AND GENOTYPIC DESCRIPTION OF VOUCHERS USED IN STUDY OF HYBRIDIZATION OF *TAMARIX RAMOSISSIMA* AND *T. CHINENSIS* (SALT CEDAR) AND *T. APHYLLA* (ATHEL).

Plant DNA #	Leaf morphology	<i>trn</i> G-S chloroplast genotype	Chloroplast genotype similar to:	<i>pepC</i> nuclear genotype	Nuclear genotype similar to:	Voucher #	Location
USA							
3111	saltcedar	A	saltcedar	1/1	saltcedar	<i>Gaskin 3111</i>	Gila River
3119	saltcedar	D	?	1/1	saltcedar	<i>Gaskin 3119</i>	Gila River
3122	saltcedar	Q	athel	2/2	saltcedar	<i>Gaskin 3122</i>	Gila River
4103	saltcedar	G	saltcedar	12/50	saltcedar/?	<i>Barnes s.n.</i>	Lake Mead
4105	saltcedar	A	saltcedar	1/50	saltcedar/?	<i>Barnes s.n.</i>	Lake Mead
4108	saltcedar	A	saltcedar	1/2	saltcedar	<i>Barnes s.n.</i>	Lake Mead
3121	athel	Q	athel	61/62	athel	<i>Gaskin 3121</i>	Gila River
4100	athel	Q	athel	61/61	athel	<i>Barnes s.n.</i>	Lake Mead
4102	athel	Q	athel	61/61	athel	<i>Barnes s.n.</i>	Lake Mead
4104	athel	Q	athel	61/61	athel	<i>Barnes s.n.</i>	Lake Mead
4098	intermediate	Q	athel	2/59	hybrid	<i>Barnes s.n.</i>	Lake Mead
4101	intermediate	Q	athel	2/62	hybrid	<i>Barnes s.n.</i>	Lake Mead
4106	intermediate	Q	athel	2/62	hybrid	<i>Barnes s.n.</i>	Lake Mead
4107	intermediate	Q	athel	2/62	hybrid	<i>Barnes s.n.</i>	Lake Mead
3113	intermediate	Q	athel	60/63	hybrid	<i>Gaskin 3113</i>	Gila River
3116	intermediate	Q	athel	1/62	hybrid	<i>Gaskin 3116</i>	Gila River
3120	intermediate	Q	athel	1/62	hybrid	<i>Gaskin 3120</i>	Gila River
4494	intermediate	Q	athel	2/62	hybrid	<i>Shafroth B1</i>	Blythe
4495	intermediate	Q	athel	1/62	hybrid	<i>Shafroth B2</i>	Blythe
4496	intermediate	Q	athel	2/65	hybrid	<i>Shafroth B3</i>	Blythe
4497	intermediate	Q	athel	2/65	hybrid	<i>Shafroth B4</i>	Blythe
4498	intermediate	Q	athel	2/65	hybrid	<i>Shafroth B5</i>	Blythe
4499	intermediate	Q	athel	2/62	hybrid	<i>Shafroth B6</i>	Blythe
4500	intermediate	Q	athel	2/65	hybrid	<i>Shafroth B7</i>	Blythe
Australia							
2043	athel	Q	athel	61/62	athel	<i>Gavin 2</i>	Finke River
2044	athel	Q	athel	61/61	athel	<i>Gavin 3</i>	Finke River
2045	athel	Q	athel	61/61	athel	<i>Gavin 4</i>	Finke River
2046	athel	Q	athel	61/61	athel	<i>Gavin 5</i>	Finke River
2047	athel	Q	athel	61/62	athel	<i>Gavin 6</i>	Finke River
2048	athel	Q	athel	60/61	athel	<i>Gavin 7</i>	Finke River

There were eight intermediates within a ca. 0.05 ha area at the upland-bottomland interface, adjacent to saltcedars. There are hundreds of athels planted at abandoned settlements within about 5 km of this site, and at least one other intermediate was observed ca. 2.5 km from the group of eight intermediates from which we collected samples, less than 100 m from a large population of saltcedars.

The third population is over 400 km from the Lake Mead site and over 160 km from the Blythe site, along the Gila River approximately 4 km downstream of Painted Rock dam in Maricopa County, AZ (ca. 33°5'2"N lat., 113°3'19"W long., elev. 160 m). The morphologically intermediate plants are located near athels that were most likely planted as a wind break in an agricultural setting near the mouth of an ephemeral wash, and numerous saltcedars are found nearby. *Tamarix* plants within three vegetation patches that contained at least some of the morphologically intermediate plants were censused. The area of the patches to-

taled 2.1 hectares, and a total of 139 athels, 134 saltcedars, and 28 intermediates were counted. Other nearby patches of saltcedar did not appear to contain intermediates; however, our survey was not comprehensive. Age estimates of the intermediates range from 8–13 years based on counts of annual rings on stem cross-sections cut from the main stem (below surface branch points) of six individuals.

Australian athel samples were provided from Horseshoe Bend on the Finke River, Northern Territory (six separate sites, ca. 25°13'S lat., 134°11'E long.). Representative vouchers, listed in Table 1, have been deposited at the Missouri Botanical Garden herbarium (MO).

Morphology

Identity of specimens was determined using the key to species in Baum (1978) which covers the genus worldwide. Morphological intermediacy was determined from leaf morphology alone because

very few plants were fertile at the time of collection.

Tissue Sampling

Leaf tissue samples were collected from 24 *Tamarix* individuals from the three USA sites. Of these, six have sessile leaves typical of saltcedar (e.g., *T. ramosissima* or *T. chinensis*), four have vaginate leaves typical of athel (*T. aphylla*), and 14 have morphologically intermediate leaves that strongly clasp the stem, but are not completely vaginate. Leaf tissue was also taken from the six Australian athel samples.

DNA Isolation, PCR Amplification and Sequencing

Fresh, silica dried tissue was used for DNA extraction. Genomic DNA was isolated using a modified CTAB method (Hillis et al. 1996). PCR amplification of the chloroplast intergenic region between the *trn S* (GCU) and *trn G* (UCC) genes utilized the primer pair *trn S* (GCU) (5'-GCCGCTTTAGTCCACTCAGC-3') and *trn G* (UCC) (5'-GAACGAATCACACTTTTACCAC-3') of Hamilton (1999) with the following cycling conditions: 95°C (2 min); 30 cycles of 95°C (1 min), 55°C (1min), 72°C (2 min); and then 32°C (5 min). The nuclear fourth *pepC* (phosphoenolpyruvate carboxylase) intron region was amplified by PCR using primer pair PPCL1 (forward) (5'-GTCCCTAAGTTTCTGCGTCG-3') and PPCL2 (reverse) (5'-CTTCAGGTGTTACTCTTGGG-3') (designed by J.G.) with the following cycling conditions: 95°C (2 min); 30 cycles of 95°C (1 min), 50°C (1 min), 72°C (2 min); and then 32°C (5 min). A 50-μl reaction was performed for each individual, and PCR products were purified using QIAquick PCR Purification kit (Qiagen, Valencia, CA). Purified templates were sequenced in two directions by using either an ABI 373A or a Beckman CEQ 2000XL automated sequencer, using the same primers listed above. Sequences generated in this study are available on GenBank, and accession numbers are listed in Table 2. For heterozygous nuclear sequences, haplotypes were first inferred using "haplotype subtraction" (Clark 1990). To verify our estimation of nuclear haplotypes not found in the homozygotic states, we sequenced cloned PCR products of selected heterozygotic plants. Cloning was done on purified template with TOPO TA Cloning Kit for Sequencing (Invitrogen, Carlsbad, CA) using standard protocol for chemically competent cells. Cell cultures were grown on kanamycin plates, and individual colonies were picked from the plates, amplified, purified, and then sequenced using the protocols listed above. Haplotype sequences were manually aligned using the software Se-Al (Rambaut 1996). The alignment is available upon request from the first author. Most parsimonious haplotype networks were created by hand. Insertion/deletion

TABLE 2. GENBANK ACCESSION NUMBERS FOR *TAMARIX* HAPLOTYPES.

Nuclear haplotypes	
1	AY090385
2	AY090386
12	AY090396
50	AY090434
59	AY672672
60	AY672673
61	AY672671
62	AY672674
63	AY672675
65	AY672676
Chloroplast haplotypes	
A	AF490798
D	AF539998
Q	AF490795
G	AF490782

events, no matter what size, were treated as one mutational event (one evolutionary step).

Testing Seed Viability

Seeds were plated between discs of filter paper in a standard Petri dish, and moistened with distilled H₂O. Petri dishes were subjected to 24°C and 14 hrs of light per day. After 48 hours, seeds that turned green and had a root radicle emerge from the seed coat were considered viable.

RESULTS

Morphology

We attempted to use the species key (Baum 1978) for the few plants with intermediate leaf morphology that were fertile, but failed to identify them to species with this method. Their leaf morphology most closely resembled *T. bengalensis* Baum or *T. indica* Willd. The morphological intermediates differ from *T. bengalensis* in having conspicuous salt glands and 1 mm narrowly ovate sepals rather than 1.25–1.5 mm orbicular to broadly ovate sepals, and they differ from *T. indica* in having entire sepals rather than deeply incised-denticulate sepals, especially towards the sepal apex. The 1.75 mm obovate petals of the few fertile intermediates most closely resemble those of *T. ramosissima*.

Chloroplast Marker

The *trn S* (GCU)–*trn G* (UCC) intergenic region sequenced is 1001 bases in length. The plants that morphologically resemble saltcedar have chloroplast haplotypes A, D, G, or Q. In a previous study (Gaskin 2003) haplotype A was found in 77% (n = 23) of USA saltcedars, D was found in 3% (n = 1) (but this haplotype is most often found in *T. parviflora* DC. in the USA (Gaskin and Schaal 2003)), and G was found in 17% (n = 5) (usually found in the horticultural *T. ramosissima* 'Pink

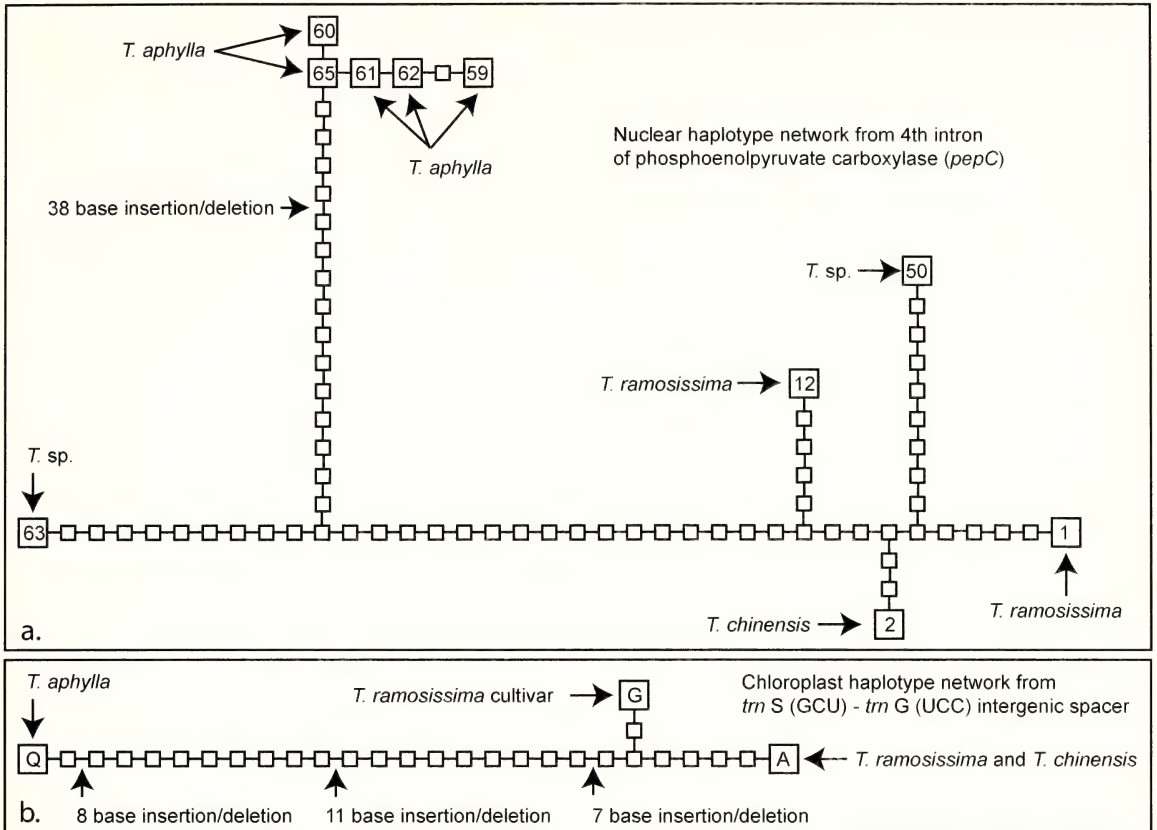


FIG. 3. Haplotype networks (gene genealogies) of the nuclear phosphoenolpyruvate carboxylase (*pepC*) 4th intron region (a) and chloroplast *trn S* (GCU)–*trn G* (UCC) intergenic region (b). Boxes with numbers or letters represent haplotypes (alleles) recovered. The smaller empty boxes represent intermediate haplotypes not recovered in this analysis. Lines separating boxes represent a single point mutation or insertion/deletion event.

Cascade' cultivar (Gaskin 2003)). Athel samples from the USA and Australia all contain the haplotype Q, which is 26 (2.6%) mutational differences (including 7, 8, and 11 base insertion/deletions) from the saltcedar haplotype A (Fig. 3). The samples with intermediate morphologies all contain haplotype Q, which is also found in all athel in this study. Chloroplast haplotype names follow Gaskin (2003).

Nuclear Marker

The *pepC* intron region sequenced is approximately 900 bases in length. Initial direct sequencing of PCR product found 13 genotypic combinations (see Table 3), three of which are homozygotic (1/1, 2/2, and 61/61). From the direct sequencing data we infer that there are ten haplotypes (1, 2, 12, 50, 59, 60, 61, 62, 63, and 65). The existence of haplotypes 1, 2, and 61 are obvious from their presence in homozygotic plants. To verify the sequences of other inferred haplotypes found only in heterozygotes, we sequenced cloned product from specimens 3113 (genotype 60/63), 3121 (61/62), 4098 (2/59), 4103 (12/50), 4105 (1/50), 4106 (2/62),

4108 (1/2), and 4500 (2/65). In each case the cloned product sequences exactly matched one of the inferred haplotypes from that plant.

The plants that morphologically resemble saltcedar contain nuclear genotypes 1/1, 2/2, 1/2, 1/50, and 12/50. The first four genotypes are identical to those found in 21% (n = 32), 19% (n = 30), 21% (n = 33), and 1% (n = 2) (respectively) of the USA saltcedars sampled in a previous study (Gaskin and Schaal 2002). Genotype 12/50 was not found in that study, but is composed of two haplotypes that were found in 10% and 1% (respectively) of the USA saltcedars (Gaskin and Schaal 2002). Nuclear haplotypes designations follow Gaskin and Schaal (2002).

The USA athel specimens have genotypes 60/61, 61/61, and 61/62, and the haplotypes involved in these genotypic combinations cluster tightly on the gene tree (Fig. 3), differing by only three mutations, and are at least 39 (4.3%) mutational differences (including a prominent 38 base insertion/deletion) away from haplotypes found in plants that morphologically resemble the saltcedars (1, 2, 12, and 50). The Australian athel samples have genotypes 60/

TABLE 3. NUCLEOTIDE STATE AT VARIABLE LOCI OF THE NUCLEAR FOURTH *PEPC* (PHOSPHOENOLPYRUVATE CARBOXYLASE) INTRON REGION OF *TAMARIX* SPP.

Leaf morphology	Nuclear geno-type	Nucleotide site #													
		1	13	108	109	112	150	152	162	189	191	197	245	264	
Saltcedar	1/1	G	T	T	G	A	A	A	A	T	T	T	C	A	
Saltcedar	1/2	G	T	T	G	A	A	A	A	T	T	T	C	A	
Saltcedar	1/50	G	T	T	G	A	A	A	A	T	T	C/T	C	A	
Saltcedar	2/2	G	T	T	G	A	A	A	A	T	T	T	C	A	
Saltcedar	12/50	G	T	T	G	A	A	A	A	T	T	C/T	C	A	
Intermediate	1/62	A/G	C/T	C/T	C/G	A/C	A/C	A/G	A/C	T	T	T	C/G	A/C	
Intermediate	2/59	A/G	C/T	C/T	C/G	A/C	A/C	A/G	A/C	A/T	A/T	T	C/G	A/C	
Intermediate	2/62	A/G	C/T	C/T	C/G	A/C	A/C	A/G	A/C	T	T	T	C/G	A/C	
Intermediate	2/65	A/G	C/T	C/T	C/G	A/C	A/C	A/G	A/C	T	T	T	C/G	A/C	
Intermediate	60/63	A	C	C/T	C	C	C	A/G	A/C	T	T	T	G	C	
Athel	60/61	A	C	C	C	C	C	G	C	T	T	T	G	C	
Athel	61/61	A	C	C	C	C	C	G	C	T	T	T	G	C	
Athel	61/62	A	C	C	C	C	C	G	C	T	T	T	G	C	

Leaf morphology	Nuclear geno-type	Nucleotide site #													
		266	277	289	297	300	343	359	397	400	410	431	450	453	
Saltcedar	1/1	A	A	A	G	G	C	C	A	C	A	T	T	T	
Saltcedar	1/2	A/T	A/G	A	G	G	C	C	A	C	A/T	A/T	T	T	
Saltcedar	1/50	A	G	A	G	G	C	C	A	C	A	A/T	A	A/T	
Saltcedar	2/2	T	G	A	G	G	C	C	A	C	T	A	—	T	
Saltcedar	12/50	A	G	A	G	A/G	C	C	A	C	A	A	A	A/T	
Intermediate	1/62	A	A/G	A/C	G/T	A/G	C/T	C/T	A/C	C/T	A/C	A/T	T	T	
Intermediate	2/59	T	G	A/C	G/T	A/G	C/T	C/T	A/C	C/T	T	A	—	T	
Intermediate	2/62	T	G	A/C	G/T	A/G	C/T	C/T	A/C	C/T	T	A	—	T	
Intermediate	2/65	T	G	A/C	G/T	A/G	C/T	C/T	A/C	C/T	T	A	—	T	
Intermediate	60/63	A	G	C	T	A	C/T	T	C	C/T	A/C	A	A/T	T	
Athel	60/61	A	G	C	T	A	T	T	C	T	C	A	T	T	
Athel	61/61	A	G	C	T	A	T	T	C	T	C	A	T	T	
Athel	61/62	A	G	C	T	A	T	T	C	T	C	A	T	T	

Leaf morphology	Nuclear geno-type	Nucleotide site #													
		455	460	466	496	498	503	514	554	555	568	574	576	587	
Saltcedar	1/1	T	A	T	T	G	A	C	T	A	A	C	C	C	
Saltcedar	1/2	T	A	T	C/T	A/G	A	C	T	A	A/G	C	C	C	
Saltcedar	1/50	A/T	A	T	C/T	A/G	A/T	C	T	A	A	C	C	C	
Saltcedar	2/2	T	A	T	C	A	A	C	T	A	G	C	C	C	
Saltcedar	12/50	A/T	A	G/T	C/T	A	A/T	C	T	A	A	C	C	C	
Intermediate	1/62	T	A	T	T	A/G	A	C/G	C/T	A/G	A	C/T	C	C/T	
Intermediate	2/59	T	A	T	C	A	A	C/G	C/T	A/G	A	C/T	C	C/T	
Intermediate	2/62	T	A	T	C	A	A	C/G	C/T	A/G	A	C/T	C	C/T	
Intermediate	2/65	T	A	T	C	A	A	C/G	C/T	A/G	A	C/T	C	C/T	
Intermediate	60/63	T	A/G	T	T	A	A	G	C/T	A/G	A	C/T	A/C	T	
Athel	60/61	T	A	T	T	A	A	G	C	G	A	T	C	T	
Athel	61/61	T	A	T	T	A	A	G	C	G	A	T	C	T	
Athel	61/62	T	A	T	T	A	A	G	C	G	A	T	C	T	

61, 61/61, and 61/62, which are all identical to athel genotypes found in the USA.

The samples with intermediate morphologies all have heterozygous genotypes for this diploid marker. Some haplotypes found in these heterozygotes (1, 2, 60, and 62) match exactly those found in the athel and saltcedar specimens, while haplotypes 59, 63, and 65 do not. Haplotypes 59 and 65 are most likely athel haplotypes, as they are very close (one

mutation) to the cluster of athels on the gene tree. The taxonomic origin of haplotype 63 is unknown, as it is distant from all other haplotypes and taxa in this study. The morphologically intermediate genotypes are 1/62 (*T. ramosissima* × *T. aphylla*), 2/62 (*T. chinensis* × *T. aphylla*), 2/59 (*T. chinensis* × *T. aphylla*?), 2/65 (*T. chinensis* × *T. aphylla*?), and 63/60 (*T. sp.* × *T. aphylla*). None of the genotypes found in morphologically intermediate

TABLE 3. CONTINUED.

Leaf morphology	Nuclear geno-type	Nucleotide site #												
		588	610	615	628	636	637	686	696	697	710	711	712	713
Saltcedar	1/1	A	T		A	C	G	G	T	G	C	T	G	A
Saltcedar	1/2	A	T	T	A	C	G	G	T	G	C	T	G	A
Saltcedar	1/50	A	T	T	A/-	C/T	G	G	T	G	C	T	G	A
Saltcedar	2/2	A	T	T	A	C	G	G	T	G	C	T	G	A
Saltcedar	12/50	A	T	T	A/-	C/T	G	G	T	G	C	T	G	A
Intermediate	1/62	A/G	C/T	T	A	C	G	A/G	T	G/T	C/-	T/-	G/-	A/-
Intermediate	2/59	A/G	C/T	T	A	C	G	A/G	T	G/T	C/-	T/-	G/-	A/-
Intermediate	2/62	A/G	C/T	T	A	C	G	A/G	T	G/T	C/-	T/-	G/-	A/-
Intermediate	2/65	A/G	C/T	T	A	C	G	A/G	T	G/T	C/-	T/-	G/-	A/-
Intermediate	60/63	G	C/T	C/T	A	C	A/G	A	C/T	G/T	C/-	T/-	G/-	A/-
Athel	60/61	G	C	T	A	C	G	A	T	T	—	—	—	—
Athel	61/61	G	C	T	A	C	G	A	T	T	—	—	—	—
Athel	61/62	G	C	T	A	C	G	A	T	T	—	—	—	—

Leaf morphology	Nuclear geno-type	Nucleotide site #												
		714	715	716	717	718	719	720	721	722	723	724	725	726
Saltcedar	1/1	A	G	C	T	G	A	T	A	T	G	T	T	G
Saltcedar	1/2	A	G	C	T	G	A	T	A	T	G	T	T	G
Saltcedar	1/50	A	G	C	T	G	A	T	A	T	G	T	T	G
Saltcedar	2/2	A	G	C	T	G	A	T	A	T	G	T	T	G
Saltcedar	12/50	A	G	C	T	G	A	T	A	T	G	T	T	G
Intermediate	1/62	A/-	G/-	C/-	T/-	G/-	A/-	T/-	A/-	T/-	G/-	T/-	T/-	G/-
Intermediate	2/59	A/-	G/-	C/-	T/-	G/-	A/-	T/-	A/-	T/-	G/-	T/-	T/-	G/-
Intermediate	2/62	A/-	G/-	C/-	T/-	G/-	A/-	T/-	A/-	T/-	G/-	T/-	T/-	G/-
Intermediate	2/65	A/-	G/-	C/-	T/-	G/-	A/-	T/-	A/-	T/-	G/-	T/-	T/-	G/-
Intermediate	60/63	A/-	G/-	C/-	T/-	G/-	A/-	T/-	A/-	T/-	G/-	T/-	T/-	G/-
Athel	60/61	—	—	—	—	—	—	—	—	—	—	—	—	—
Athel	61/61	—	—	—	—	—	—	—	—	—	—	—	—	—
Athel	61/62	—	—	—	—	—	—	—	—	—	—	—	—	—

Leaf morphology	Nuclear geno-type	Nucleotide site #												
		727	728	729	730	731	732	733	734	735	736	737	738	739
Saltcedar	1/1	T	G	G	C	T	T	T	T	A	A	T	A	T
Saltcedar	1/2	T	G	G	C	T	T	T	T	A	A	T	A	T
Saltcedar	1/50	T	G	G	C	T	T	T	T	A	A	T	A	T
Saltcedar	2/2	T	G	G	C	T	T	T	T	A	A	T	A	T
Saltcedar	12/50	T	G	G	C	T	T	T	T	A	A	T	A	T
Intermediate	1/62	T/-	G/-	G/-	C/-	T/-	T/-	T/-	T/-	A/-	A/-	T/-	A/-	T/-
Intermediate	2/59	T/-	G/-	G/-	C/-	T/-	T/-	T/-	T/-	A/-	A/-	T/-	A/-	T/-
Intermediate	2/62	T/-	G/-	G/-	C/-	T/-	T/-	T/-	T/-	A/-	A/-	T/-	A/-	T/-
Intermediate	2/65	T/-	G/-	G/-	C/-	T/-	T/-	T/-	T/-	A/-	A/-	T/-	A/-	T/-
Intermediate	60/63	T/-	G/-	G/-	C/-	T/-	T/-	T/-	T/-	A/-	A/-	T/-	A/-	T/-
Athel	60/61	—	—	—	—	—	—	—	—	—	—	—	—	—
Athel	61/61	—	—	—	—	—	—	—	—	—	—	—	—	—
Athel	61/62	—	—	—	—	—	—	—	—	—	—	—	—	—

plants were ever detected in a previous analysis of 269 saltcedars worldwide (Gaskin and Schaal 2002).

Viability of Seed with Intermediate Morphology

One plant (*Shafroth B1*) from near Walters Camp, CA was in seed on December 12, 2003. Seeds were plated three days after being collected, and nine of the 239 seeds (3.8%) exhibited viability.

DISCUSSION

The nuclear genotypes found in the morphologically intermediate plants are most often exact combinations of athel and saltcedar haplotypes, supporting our first hypothesis of hybridization. The presence of chloroplast sequence in the morphologically intermediate plants that is identical to that found in all specimens of athel also supports the hypothesis of hybridization. The DNA data do not support the hypothesis of morphologically inter-

TABLE 3. CONTINUED.

Leaf morphology	Nuclear geno-type	Nucleotide site #												
		740	741	742	743	744	745	746	747	778	782	797	798	821
Saltcedar	1/1	T	G	T	A	T	A	C	T	G	A	G	G	T
Saltcedar	1/2	T	G	T	A	T	A	C	T	G	A	G	G	T
Saltcedar	1/50	T	G	T	A	T	A	C	T	G	A	G	G	T
Saltcedar	2/2	T	G	T	A	T	A	C	T	G	A	G	G	T
Saltcedar	12/50	T	G	C/T	A	T	A	C	T	G	A	C/G	G	T
Intermediate	1/62	T/-	G/-	T/-	A/-	T/-	A/-	C/-	T/-	A/G	A	C/G	A/G	C/T
Intermediate	2/59	T/-	G/-	T/-	A/-	T/-	A/-	C/-	T/-	A/G	A	C/G	A/G	C/T
Intermediate	2/62	T/-	G/-	T/-	A/-	T/-	A/-	C/-	T/-	A/G	A	C/G	A/G	C/T
Intermediate	2/65	T/-	G/-	T/-	A/-	T/-	A/-	C/-	T/-	A/G	A	C/G	A/G	C/T
Intermediate	60/63	T/-	G/-	T/-	A/-	T/-	A/-	C/-	T/-	A/G	A/G	C	A/G	C
Athel	60/61	—	—	—	—	—	—	—	—	A	A	C	A	C
Athel	61/61	—	—	—	—	—	—	—	—	A	A	C	A	C
Athel	61/62	—	—	—	—	—	—	—	—	A	A	C	A	C

Leaf morphology	Nuclear geno-type	Nucleotide site #											
		825	847	863	890	891	892	893	894	895	902	905	906
Saltcedar	1/1	G	G	G	—	—	—	—	—	—	A	C	A
Saltcedar	1/2	G	G	G	—	—	—	—	—	—	A	C	A/G
Saltcedar	1/50	G	G/T	G	—	—	—	—	—	—	A	C	A
Saltcedar	2/2	G	G	G	—	—	—	—	—	—	A	C	G
Saltcedar	12/50	G	G/T	G	—	—	—	—	—	—	A	C/T	A/G
Intermediate	1/62	G/T	G	A/G	—	—	—	—	—	—	A	C/T	A/G
Intermediate	2/59	G/T	G	A/G	—	—	—	—	—	—	A	C/T	G
Intermediate	2/62	G/T	G	A/G	—	—	—	—	—	—	A	C/T	G
Intermediate	2/65	G/T	G	A/G	—	—	—	—	—	—	A/G	C	G
Intermediate	60/63	T	G	A	A/-	T/-	A/-	T/-	G/-	A/-	G	C/T	A/G
Athel	60/61	T	G	A	—	—	—	—	—	—	A/G	C/T	G
Athel	61/61	T	G	A	—	—	—	—	—	—	A	C	G
Athel	61/62	T	G	A	—	—	—	—	—	—	A	C/T	G

mediate plants being a previously unrecorded species in the USA, as none of the morphologically intermediate plants contain chloroplast sequences different from those found commonly in other *Tamarix* species in the USA, and all morphologically intermediate plants contain at least one nuclear haplotype commonly found in a putative parental species. The seed source of the hybrid plants investigated is athel, assuming maternal inheritance of the chloroplast marker. Most angiosperms exhibit this inheritance pattern (Reboud and Zeyl 1994), however, cpDNA can occasionally be inherited from either parent or biparentally, as in *Turnera* L. (Shore and Triassi 1998). Plastid inheritance in the family Tamaricaceae has not been investigated.

The three different Australian athel nuclear marker genotypes are identical to athel genotypes found in the USA. Perhaps with a more variable marker, Australian specimens could be distinguished from USA specimens, but at this point, given the genetic similarity and the observations of seedling establishment on Lake Mead (Barnes 2003), we should assume that the athel present in the USA, under certain conditions, could invade more sites. However, athel has been present for sev-

eral decades in the western USA at a variety of sites and has not yet spread extensively.

The saltcedar *Tamarix chinensis* (represented by nuclear haplotype 2) and athel are allopatric in Eurasia, with haplotype 2 native to eastern China (Gaskin and Schaal 2002) and athel ranging from northern Africa to Pakistan (Baum 1978). Thus, we expect that the hybrids between these two historically allopatric species are novel. The ranges of the saltcedar *T. ramosissima* and athel overlap slightly in areas of Asia, but hybrids of athel and saltcedar are not mentioned in the Eurasian literature, nor did the authors note them while collecting in areas of Asia where the two species are sympatric.

There is genetic variation within the hybrids, indicated by the five different nuclear genotypes found in our small sample of 14 plants of intermediate morphology. Crosses between hybrids or backcrosses with either parent could increase the number of genotypes, providing additional evolutionary opportunities for the success of the hybrid lineage. One of the plants (*Gaskin 3122*), classified as saltcedar according to its morphology and nuclear genotype (Q). The sample was re-sequenced to con-

firm this result. The presence of the athel chloroplast haplotype Q in this plant must have occurred through backcrossing of a hybrid with a saltcedar or another hybrid plant (e.g., crossing Q-60/60 with A-2/2, resulting in the hybrid Q-60/2, then backcrossing the hybrid with a saltcedar A-2/2, resulting in a plant with chloroplast-nuclear genotype Q-2/2).

Our observed 3.8% viability of seed from one of the hybrids is low compared to up to 98% viability for *T. ramosissima* and 100% viability for *T. aphylla* when germinated with tap water within a week after collection (Horton 1960; Waisel 1960). However, we do not know how long our seeds had been mature and attached to the plant before being collected. Seed viability of both species is known to decrease with storage time after collection (Horton 1960; Waisel 1960), and saltcedar seed viability can decrease to as low as 20% after 10 weeks under field conditions (Horton 1960).

Even with a low viability rate, a hybrid could still produce a substantial number of viable seeds, as mature saltcedars have been estimated to produce several hundred thousand seeds in a single growing season (Merkel 1957). However, besides the molecular evidence of backcrossing and the one plant producing seed near Walters Camp, CA, we found no other verification that the hybrids produce viable seed. On June 19, 2003, some Lake Mead hybrids were producing flowers, but none of them appeared to have set seed. At the same time, there were no flowers (pollen sources) on nearby athel or saltcedars (J.G. personal observation). The lower water levels of Lake Mead have reduced flowering and seed set in athel (Barnes 2003). If the lake level is raised, or if athel establish at lower shoreline elevations (with better access to soil moisture), then increased flowering may provide more opportunities for hybridization or backcrossing. On August 15, 2003, hybrids at the Gila River site had mature flowers, but none appeared to have successfully set seed (R. Laugharn, personal observation).

Athel is presumably limited in latitudinal range by frost intolerance, and is rarely seen north of southern Utah (approximately 38°N lat.; Welsh et al. 1993) in the Colorado River watershed, or north of Las Cruces, NM (approximately 33°N lat.; Allred 2002) in the Rio Grande watershed. Saltcedar does not have such severe frost limitations and is found as far north as 52°N lat. in Asia (Baum 1978), and naturalized as far north as 48°N lat. in North Dakota (specimen *Mayer 20* (USDA-ARS-NPARK)), with cultivated plants extending into Canada (Kartesz and Meacham 1999; Pearce and Smith 2003). There are many locations where saltcedars and athel are sympatric in the USA, and the authors have searched for hybrids at several of these locations in California, Arizona, New Mexico, and Texas. However, the hybrid has only been confirmed from the three areas mentioned in this paper. Apparent morphological intermediates were

also observed within the Colorado River bottomland east of Needles, CA (elevation ca. 190 m) but were not collected nor included in this study.

The flowering phenologies of the parental species are different, but overlapping. The saltcedars flower from early spring to late fall, while athel flowers later in the year, from the end of summer to early winter (Baum 1978) (also see Barnes 2003 for athel phenology in the Lake Mead area), leaving ample opportunity for gene flow between saltcedars and athel. The late-season seed dispersal phenology of athel may contribute to the apparently infrequent seed reproduction of this species, and therefore of the hybrid we have described here, given the apparent maternal origin of the hybrid seed. Successful *Tamarix* seed germination generally requires bare, moist substrates, which are less likely to occur on river floodplains in the Sonoran or Mojave deserts in fall months than other times of the year, except perhaps following flooding associated with occasional tropical storms (Ely et al. 1994). Further, for seedling establishment to occur in North America, fall season germinants need to survive the winter months. Although low temperatures rarely drop below freezing at the sites we studied, we speculate that an inability to over winter successfully may inhibit establishment of hybrid seedlings.

Even though it is unclear if the new hybrids can commonly reproduce by seed, one of the parental species is an athel that has chloroplast and nuclear marker genotypes identical to those invading Australia, and some of the other parental species are genetically identified as the most common invasive saltcedars in the USA (genotypes 1/1, 1/2, and 2/2 (Gaskin and Schaal 2002)). The tree habit of athel, combined with aggressive characteristics of the shrub saltcedar, could potentially produce an invader that is able to compete in ways different from saltcedar with native and naturalized riparian vegetation (e.g., increased height, increased phenological range, resistance to natural and imported enemies, etc.). On the other hand, genetic combinations associated with hybridization can often result in less competitive offspring, and failure of hybrid lineages in natural settings is undoubtedly underestimated due to our inability to detect such events. We suggest that the new *Tamarix* hybrids we report here should be investigated further for pollen and seed viability, and closely monitored for their ability to spread.

ACKNOWLEDGMENTS

The authors thank J. Gavin, E. Powell, and P. Barnes for sending plant samples and S. Parsons for leaf images. G. T. Auble, J. M. Friedman, and T. Shanower provided helpful comments on an earlier version of the manuscript. This research was supported by USDA Cooperative State Research, Education, and Extension Service Grant #2000-00836 to B. Schaal and J. Gaskin, and EPA Science To Achieve Results (STAR) graduate fellowship to J. Gaskin, the Mellon Foundation support of Missouri Botanical Gar-

den graduate students, and post-doctoral funding for J. Gaskin from the USDI BLM Montana/Dakotas.

LITERATURE CITED

- ALLRED, K. W. 2002. Identification and taxonomy of tamarisk (Tamaricaceae) in New Mexico. *Desert Plants* 18:26–32.
- BARNES, P. L. 2003. Reproductive and population characteristics of *Tamarix aphylla* at Lake Mead National Recreation Area, Nevada. M.S. thesis. University of Nevada Las Vegas, Las Vegas, NV.
- BAUM, B. R. 1967. Introduced and naturalized tamarisks in the United States and Canada. *Baileya* 15:19–25.
- . 1978. The genus *Tamarix*. Israel Academy of Sciences and Humanities, Jerusalem, Israel.
- CLARK, A. G. 1990. Inference of haplotypes from PCR-amplified samples of diploid populations. *Molecular Biology and Evolution* 7:111–112.
- CRINS, W. J. 1989. The Tamaricaceae of the southeastern United States. *Journal of the Arnold Arboretum* 70: 403–425.
- ELLSTRAND, N. C. AND K. A. SCHIERENBECK. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Science* 97:7043–7050.
- ELY, L. L., Y. ENZEL, AND D. R. CAYAN. 1994. Anomalous North Pacific atmospheric circulation and large winter floods in the southwestern United States. *Journal of Climate* 7:977–987.
- FULLER, M. 1998. Athel Pine. *Agnote* 539 No. F61. Available at: <http://www.ipe.nt.gov.au/whatwedo/weeds/pdf/tamarixaphylla.pdf>. Northern Territory Government, Department of Business, Industry and Resource Development, Alice Springs, Australia.
- GASKIN, J. F. 2003. Molecular systematics and the control of invasive plants: a case study of *Tamarix* (Tamaricaceae). *Annals of the Missouri Botanical Garden* 90:109–118.
- AND B. A. SCHAAL. 2002. Hybrid *Tamarix* widespread in US invasion and undetected in native Asian range. *Proceedings of the National Academy of Sciences of the United States of America* 99:11256–11259.
- AND B. A. SCHAAL. 2003. Molecular phylogenetic investigation of US invasive *Tamarix*. *Systematic Botany* 28:86–95.
- GRIFFIN, G. F., D. M. STAFFORD SMITH, S. R. MORTON, G. E. ALLAN, AND K. A. MASTERS. 1989. Status and implications of the invasion of tamarisk (*Tamarix aphylla*) on the Finke River, Northern Territory, Australia. *Journal of Environmental Management* 29:297–315.
- HAMILTON, M. 1999. Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. *Molecular Ecology* 8:521–523.
- HILLIS, D. M., B. K. MABLE, A. LARSON, S. K. DAVIS, AND E. A. ZIMMER. 1996. *Molecular systematics*. Sinauer, Sunderland, MA.
- HORTON, J., F. MOUNTS, J. M. KRAFT. 1960. Seed germination and seedling establishment of phreatophyte species. Rocky Mountain Forest and Range Experiment Station, Ft. Collins, CO.
- KARTESZ, J. T. AND C. A. MEACHAM. 1999. *Synthesis of the North American flora*. North Carolina Botanical Garden, Chapel Hill, NC.
- KOWARIK, I. 1995. Time lags in biological invasions with regard to the success and failure of alien species. Pp. 15–38 in P. Pyšek, K. Prach, M. Rejmánek, and M. Wade (eds.), *Plant invasions, general aspects and special problems*. SPB Academic Publishers, Amsterdam, the Netherlands.
- LOUDA, S. M., D. KENDALL, J. CONNOR, AND D. SIMBERLOFF. 1997. Ecological effects of an insect introduced for the biological control of weeds. *Science* 277: 1088–1090.
- MERKEL, D. AND H. HOPKINS. 1957. Life history of salt cedar (*Tamarix gallica* L.). *Transactions of the Kansas Academy of Science* 60:360–369.
- MEYERS-RICE, B. 1997. Weed notes: *Tamarix aphylla*. Available at: <http://tncweeds.ucdavis.edu/moredocs/tamaph01.pdf>. The Nature Conservancy, Davis, CA.
- PEARCE, C. M. AND D. G. SMITH. 2003. Saltcedar: distribution, abundance, and dispersal mechanisms, northern Montana, USA. *Wetlands* 23:215–228.
- RAMBAUT, A. 1996. Se-Al sequence alignment editor. Available at: <http://evolve.zoo.ox.ac.uk/software.html?name=Se-Al>. Department of Zoology, University of Oxford, Oxford, England.
- REBOUD, X. AND C. ZEYL. 1994. Organelle inheritance in plants. *Heredity* 72:132–140.
- RIESEBERG, L. H., M. A. ARCHER, AND R. K. WAYNE. 1999. Transgressive segregation, adaptation and speciation. *Heredity* 83:363–372.
- SHORE, J. S. AND M. TRIASSI. 1998. Paternally biased cpDNA inheritance in *Turnera ulmifolia* (Turneraceae). *American Journal of Botany* 85:328–332.
- STEBBINS, G. L. 1969. The significance of hybridization for plant taxonomy and evolution. *Taxon* 18:26–35.
- THORP, J. AND R. LYNCH. 2000. The determination of weeds of national significance. Available at: <http://www.weeds.org.au/docs/WONS/>. National Weeds Strategy Executive Committee, Launceston, Australia.
- U.S. CONGRESS, OFFICE OF TECHNOLOGY ASSESSMENT. 1993. Harmful non-indigenous species in the United States. Report # OTA-F-565. U.S. Government Printing Office, Washington, DC.
- VILA, M., E. WEBER, AND C. M. D'ANTONIO. 2000. Conservation implications of invasion by plant hybridization. *Biological Invasions* 2:207–217.
- WASEL, Y. 1960. Ecological studies on *Tamarix aphylla* (L.) Karst. I. Distribution and reproduction. *Phyton* 15:7–17.
- WELSH, S. L., N. D. ATWOOD, S. GOODRICH, AND L. C. HIGGINS (eds.), 1993. *A Utah flora*. Brigham Young University, Provo, UT.
- ZAVALA E. 2000. The economic value of controlling an invasive shrub. *Ambio* 29:462–467.